CLAIMS

What is claimed is:

1. A method of obtaining and improving the production of a functional galactose oxidase polypeptide by a host cell comprising the steps of:
   (a) providing at least one parent galactose oxidase polynucleotide encoding a parent galactose oxidase polypeptide,
   (b) altering the nucleotide sequence of the parent polynucleotide by random mutagenesis to produce a population of mutant polypeptides;
   (c) transforming host cells to express the mutant polypeptides;
   (d) screening for first-generation functional mutants produced by the host cells and having at least one modified property;
   (e) selecting at least one polynucleotide encoding a first-generation mutant as a parent polynucleotide; and
   (f) repeating a round of altering, transforming and screening steps at least once to obtain at least one other generation of one or more mutants.

2. The method of claim 1 wherein the method of random mutagenesis comprises an error-prone polymerase chain reaction.

3. The method of claim 2, wherein the error-prone polymerase chain reaction employs unbalanced nucleotide concentrations.

4. The method of claim 2, wherein the error-prone polymerase chain reaction employs manganese ions in a concentration of about 0 to about 500 μM.

5. The method of claim 2, wherein the error-prone polymerase chain reaction employs manganese ions in a concentration of about 100 μM.

6. The method of claim 2, wherein the polymerase chain reaction generates an error rate of about 1-2 mutations per polynucleotide.

7. The method of claim 2, wherein the polymerase chain reaction generates an error rate of up to about six mutations per polynucleotide.

8. The method of claim 1, wherein at least one of the altering, transforming and screening steps are changed in at least one repeated round.

9. The method of claim 1, wherein the conditions for random mutagenesis in at least one repeated round of altering, transforming and screening are different from the conditions in any other round of altering, transforming and screening.

10. The method of claim 1, wherein the host cells in at least one repeated round of altering, transforming and screening are different from the host cells in any other round of altering, transforming and screening.

11. The method of claim 10, wherein the host cells in at least one round are bacterial cells.

12. The method of claim 11, wherein the bacterial cells are *E. coli* cells.

13. The method of claim 1, wherein at least one round of altering, transforming and screening comprises screening for a property of the polypeptide that was not screened for in another round of altering, transforming and screening.

14. The method of claim 13, wherein at least one property is selected from the group consisting of enzyme activity, enzyme selectivity, enzyme stability, and enzyme yield.

15. The method of claim 1, wherein each screening step comprises screening for one or more of the biological activity of the polypeptide, the selectively of the polypeptide, the stability of the polypeptide, and the yield of expressed polypeptide.

16. The method of claim 2, wherein the error rate in the altering step of at least one round of altering, transforming and screening is about 1-2 mutations per polynucleotide, and the error rate in the altering step of at least one other round is about 4-6 mutations per polynucleotide.

17. The method of claim 2, wherein the polymerase chain reaction employs manganese ions in a concentration of about 0.35 mM.

18. The method of claim 1, wherein screening comprises pre-screening for mutant colonies using nitrocellulose membranes.

19. A polynucleotide evolved according to the method of claim 8

20. A polynucleotide encoding for a galactose oxidase which has a mutation in at least one amino acid selected from the group consisting of A3, S10, M70, P136, G195, T218, L312, N413, V494, C515, N535, N537, S550, and S610.

21. A polynucleotide encoding for a galactose oxidase which has at least one amino acid mutation selected from the group consisting of S10P, M70V, G195E, N413D, V494A, C515S, N535D, and N537D.

22. A polynucleotide encoding for a galactose oxidase which has the amino acid mutation N537D.

23. A polynucleotide encoding for a galactose oxidase which has the amino acid mutation V494A.

24. The polynucleotide of claim 23, further comprising the amino acid mutation C515S.

25. The polynucleotide of claim 23, further comprising the amino acid mutation S10P.

26. The polynucleotide of claim 23, further comprising a silent mutation at P136.

27. The polynucleotide of claim 25, further comprising a silent mutation at P136.

28. The polynucleotide of claim 23, further comprising the amino acid mutation G195E.

29. The polynucleotide of claim 28, further comprising a silent mutation in at least one of A3 and P136.

30. The polynucleotide of claim 23, further comprising the amino acid mutation N535D.

31. The polynucleotide of claim 30, further comprising a silent mutation in at least one of P136, L312, and T218.

32. The polynucleotide of claim 23, further comprising the amino acid mutation M70V.

33. The polynucleotide of claim 32, further comprising a silent mutation at P136.

34. A polynucleotide encoding for a galactose oxidase which has the amino acid mutations V494A, S10P, M70V, G195E and N535D.

35. The polynucleotide of claim 34, further comprising a silent mutation at P136.

36. A polynucleotide encoding for a galactose oxidase which has the amino acid mutation N413D.

37. The polynucleotide of claim 36, further comprising a silent mutation at S550.

38. The polynucleotide of claim 23, further comprising the amino acid mutation N413D.

39. The polynucleotide of claim 38, further comprising a silent mutation in at least one of S550 and S610.

40. A polynucleotide encoding for a galactose oxidase which has a nucleotide mutation in at least one of positions 9, 28, 208, 408, 584, 654, 830, 936, 1237, 1481, 1543, 1603, 1609, 1650, and 1830.

41. The polynucleotide of claim 40, wherein the mutation at any of positions 9, 408, 654, 936, 1650 and 1830 is a silent mutation.

42 The polynucleotide of claim 40 which has a mutation in at least one of nucleotide positions 28, 408, 654, and 1481, wherein a thymine is replaced by a cytosine.

43. The polynucleotide of claim 40, which has a mutation in at least one of nucleotide positions 1543, 1650 and 1830, wherein a thymine is replaced by an adenine.

44. The polynucleotide of claim 40, which has a mutation in at least one of nucleotide positions 206, 936, 1237, 1603, and 1609, wherein adenine is replaced by guanine.

45. A polynucleotide encoding for a galactose oxidase which has at least one nucleotide mutation in a region encompassed by nucleotides selected from the group consisting of:

(a) 1 through 30;

(b) 200 through 700;

(c) 800 through 1000; and

(d) 1200 through 1650.

46. The polynucleotide of claim 45, which has a nucleotide mutation in a region encompassed by nucleotides 1-30, wherein a thymine is replaced by a cytosine.

47. The polynucleotide of claim 45, which has a nucleotide mutation in a region encompassed by nucleotides 1450-1550, wherein a thymine is replaced by one of a cytosine and an adenine.

48. The polynucleotide of claim 45, which has a nucleotide mutation in a region encompassed by nucleotides 1200-1250, wherein an adenine is replaced by a guanine.

49. The polynucleotide of claim 45, which has a nucleotide mutation in a region encompassed by nucleotides 1600-1650, wherein an adenine is replaced by a guanine.

50. The polynucleotide of claim 45, which has a nucleotide mutation in a region proximate to and encompassing nucleotide 208, wherein an adenine is replaced by a guanine.

51. The polynucleotide of claim 45, which has a nucleotide mutation in a region proximate to and encompassing nucleotide 585, wherein a guanine is replaced by an adenine.

52. The polynucleotide of claim 45, which has a nucleotide mutation in a region proximate to and encompassing nucleotide 1543, wherein a thymine is replaced by an adenine.

53. A polynucleotide encoding for a galactose oxidase which has at least one of the nucleotide mutations A9C, T28C, A208G, T408C, G584A, T654C, A936G, A1237G, T1481C, T1543A, A1603G, A1609G, T1650A, and T1830A.

54. The polynucleotide of claim 53, which has the nucleotide mutation T1481C.

55. The polynucleotide of claim 54, further comprising the nucleotide mutation T1543A.

56. The polynucleotide of claim 54, further comprising the nucleotide mutation T408C.

57. The polynucleotide of claim 56, further comprising a nucleotide mutation selected from the group consisting of G584A, A1603G, and A208G.

58. The polynucleotide of claim 56, further comprising at least one of the nucleotide mutations A9C, A936G, and T654C.

59. The polynucleotide of claim 56, further comprising the nucleotide mutations T28C, A208G, G584A and A1603G.

60. The polynucleotide of claim 53 which has the nucleotide mutation A1237G.

61. The polynucleotide of claim 60, further comprising at least one of the nucleotide mutations selected from the group consisting of T1650A, T1830A, and T1481C.

62. The polynucleotide of claim 61, having the nucleotide mutations A1237G, T1650A, T1481C and T1830A.

63. A galactose oxidase which has a mutation in at least one amino acid selected from the group consisting of A3, S10, M70, P136, G195, T218, L312, N413, V494, C515, N535, N537, S550, and S610.

64. A galactose oxidase which has at least one of the amino acid mutations S10P, M70V, G195E, N413D, V494A, C515S, N535D, and N537D.

65. The galactose oxidase of claim 64, which has the amino acid mutation N537D.

66. The galactose oxidase of claim 64, which has the amino acid mutation V494A.

67. The galactose oxidase of claim 66, further comprising the amino acid mutation C515S.

68. The galactose oxidase claim 66, further comprising the amino acid mutation S10P.

69. The galactose oxidase of claim 66, further comprising a silent mutation at P136.

70. The galactose oxidase of claim 68, further comprising a silent mutation at P136.

71. The galactose oxidase of claim 66, further comprising the amino acid mutation G195E.

72. The galactose oxidase of claim 71, further comprising a silent mutation in at least one of A3 and P136.

73. The galactose oxidase of claim 66, further comprising the amino acid mutation N535D.

74 The galactose oxidase of claim 73, further comprising a silent mutation in at least one of P136, L312, and T218.

75. The galactose oxidase of claim 66, further comprising the amino acid mutation M70V.

76. The galactose oxidase of claim 75, further comprising a silent mutation at P136.

77. The galactose oxidase of claim 64, which has the amino acid mutations S10P, M70V, G195E, V494A and N535D.

78. The galactose oxidase of claim 77, further comprising a silent mutation at P136.

79. The galactose oxidase of claim 64, which has the amino acid mutation N413D.

80. The galactose oxidase of claim 80, further comprising a silent mutation at S550.

81. The galactose oxidase of claim 66, further comprising the amino acid mutation N413D.

82. The galactose oxidase of claim 81, further comprising a silent mutation in at least one of S550 and S610.

add a15

Add C2